

GENETIC MOSAICISM IN THE WINGS OF *HABROBRACON JUGLANDIS*

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Work in *Drosophila* has shown that cell lineage patterns in wings, legs, and antennae are not random, but that clonally related cells appear in long narrow stripes in a longitudinal direction (Bryant and Schneiderman, 1969; Bryant, 1970; Postlethwait and Schneiderman, 1971). In *Drosophila* these patterns have been demonstrated by means of genetic mosaics which arose as a result of somatic crossing-over or of ring-X chromosome elimination. Mosaics have been found in the wasp, *Habrobracon*, over the past 40 years (P. W. Whiting, 1932; A. R. Whiting, 1939, 1961) but had not been studied with respect to lineage patterns. The discovery that the mutant *ebony* in *Habrobracon* is related to both a black body color and to the production of mosaics has enabled us to plan specific experiments involving selected gene markers and to obtain large numbers of mosaics for pattern analysis (Clark, Gould, and Graham, 1971). Lineage patterns of a longitudinal type have been found for antennae and legs of *Habrobracon* (Clark, Petters, and Bryant, 1973). In the present study such patterns are reported for wings.

The two wing mutants studied here affect the shape and size of the primary and secondary wings. Investigation of the effects on wing growth and development for mosaic wings when there is an interaction of two genotypes is significant.

MATERIALS AND METHODS

In the parasitic wasp, *Habrobracon juglandis*, haploid males are produced from unfertilized eggs, and diploids, both male and female, from fertilized eggs. The haploid males are thus of gynogenetic (maternal) origin while the diploids are of zygogenetic (biparental) origin. In addition, various types of mosaic progeny are produced (Clark, Gould, and Potts, 1968). The mosaic type of present concern is one in which part of the body is zygogenetic (biparental) and the other part is androgenetic (paternal) in nuclear origin (Z + A type).

Two recessive wing mutants were used to study patterns of development, *notched wings* (*no*) and *small wings* (*sw*). Notched wings is a temperature-sensitive mutant. Kershner (1970) showed for wasps reared at four different temperatures (35°, 30°, 25°, 18° C) that the size of the wing was dependent upon the temperature at which development occurred. In contrast to the wild type which showed only slight increases in size and no change in wing shape, the *notched wings* showed progressively smaller wings with lowered temperatures (Fig. 1). *Notched wings* reared at 35° C is phenotypically indistinguishable from wild type reared at the same temperature. At lower temperatures there is a progressive loss of the distal portion of the primary wing, and of the anterior, posterior, and distal parts of the secondary wing (Fig. 1).

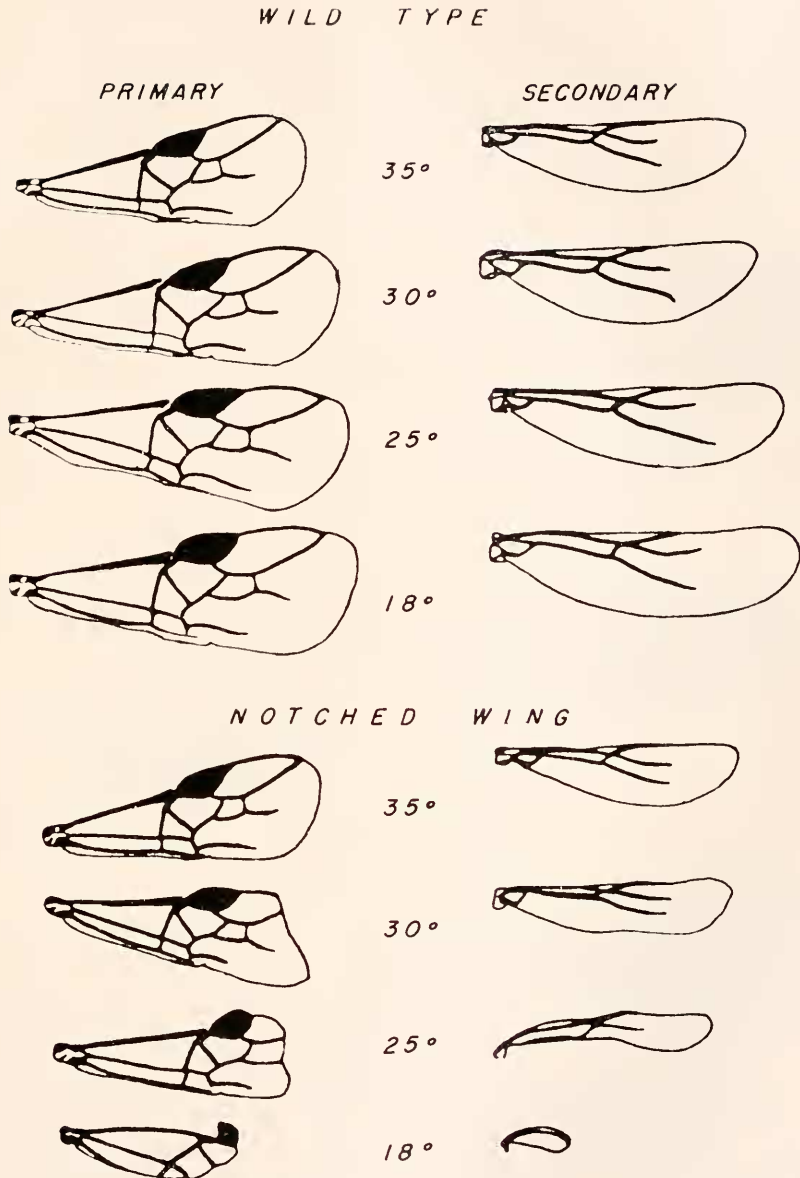


FIGURE 1. Primary and secondary wings from wild type and *notched wings* wasps reared at temperatures of 35°, 30°, 25°, and 18° C.

The *small wings* mutant has smaller primary and secondary wings than wild type (Fig. 6). In addition, wing cell size is smaller than wild type as shown by the observations that the number of microchaetae per unit area is greater in the *small wings* mutant (Clark, unpublished). Each wing microchaeta corresponds to a

single wing cell. Further, the microchaetae in the mutant are shorter than in the wild type.

Two different crosses were made. *Habrobracon* females homozygous for the recessive body color mutant *ebony* (*e*) were crossed with haploid males that were either *notched wings, honey body color* (*e/e* ♀♀ × *no ho* ♂♂) or *small wings, honey body color* (*e/e* ♀♀ × *sw ho* ♂♂). *Ebony* females cause the production of genetic mosaics among their progeny. *Honey* is a recessive body color mutant that shows light brown coloration. The honey phenotype can be recognized in wings as well as in other parts of the body. From these crosses, mosaics of the zygogenetic-androgenetic type were selected for further study. These mosaics were part wild type and part *honey*. The crosses were designed so that the wing mutant would always be found with *honey*. Thus, all wings, or parts of wings, that showed the *honey* phenotype were also either *notched wings* or *small wings* in phenotype. The mutant *honey* thus served as a marker for the wing mutants, *notched wings* and *small wings*, enabling their recognition in different regions of mosaic wings.

Adult wasps were collected. The wings were removed and mounted on slides with Permount. Drawings were made by means of a Bausch and Lomb Trisimplex Projector.

TABLE I
Wing mosaicism among mosaic Habrobracon

Cross	Mosaic wasps	Wasps with wing mosaicism			Intramosaic wings
		None	Inter	Intra	
<i>e</i> × <i>ho no</i>	120 (1.00)	59 (0.49)	30 (0.25)	31 (0.26)	39/480 (0.08)
<i>e</i> × <i>ho sw</i> *	150 (1.00)	88 (0.59)	30 (0.20)	32 (0.21)	39/600 (0.065)

* Data collected by David Wartell.

RESULTS

For the *ebony* ♀ × *notched wings, honey* ♂ cross, 120 mosaic wasps were produced. Of these, 61 showed wing mosaicism (Table I). There were 30 mosaics ("inter") defined as having individual wings either completely wild type or completely *notched wings, honey*. A bilateral mosaicism was found in which the two wings on one side were usually of one phenotype and the two wings on the other side were of the other phenotype. Among the 14 intermosaics that were found to involve two wild type wings and two *notched wings, honey*, 13 showed the presence of the wild type wings on one side and the *notched wings, honey* on the other. There were 31 mosaics ("intra") in which both the wild type and the *notched wings, honey* phenotypes were found in the same wing. Among the 480 wings that were examined from 120 mosaic wasps, 39 wings (0.08) were found to be intramosaic (Table I).

For the *ebony* ♀ × *small wings, honey* ♂ cross, 150 mosaics were produced. The frequency of the mosaic types for this cross is comparable to the one just reported (Table I). Among 19 intermosaics that were found to involve two of each phenotype, 17 were of the bilateral pattern.

Ebony × *notched wings honey*

Some examples of intermosaic wing patterns are shown in Figure 2. The mosaic 2a, which developed at 30° C, shows two right wings that are wild type and two left wings that are *honey*, *notched wings*. The left primary wing shows notching in the anterior-distal margin; the left secondary wing shown here is like the right secondary wing in shape and size. At this temperature of 30° C the *notched*

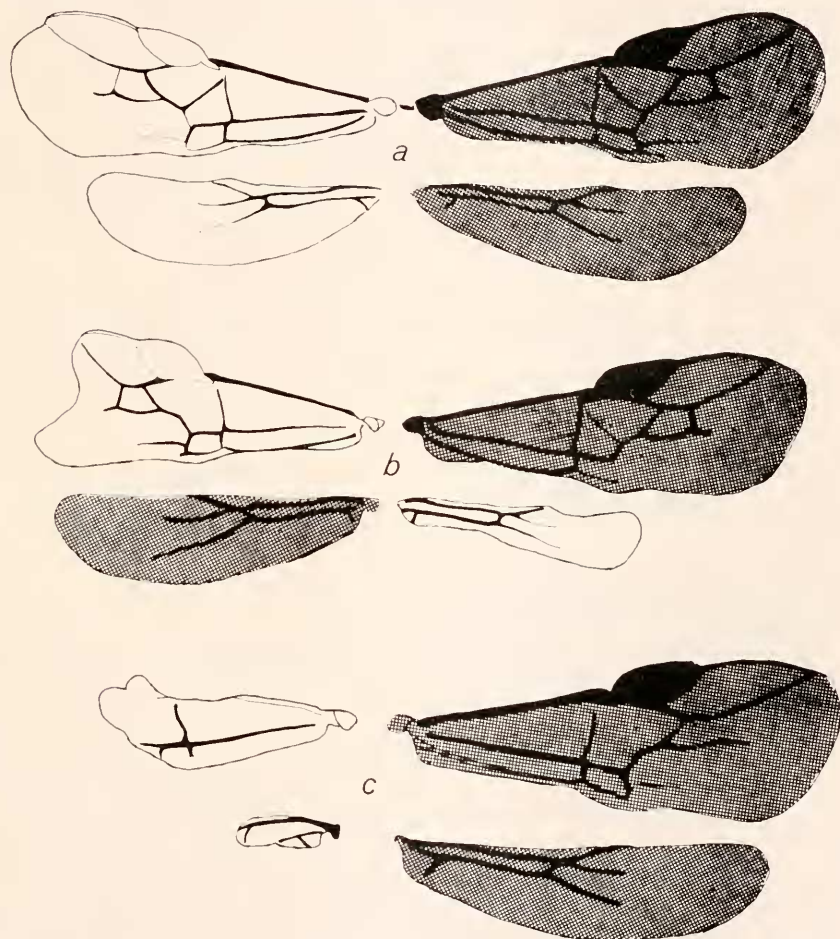


FIGURE 2. Wings from mosaic wasps. The crosshatched wings indicate the wild type phenotype and the clear wings indicate the *notched wings*, *honey* phenotype. Four wings from each of three wasps reared at 30° (a), 25° (b), and 20° (c) are shown.

wings phenotype is sometimes not expressed. The mosaic 2b, developed at 25° C, shows a left primary wing with scalloping of the distal part and a right secondary wing that is smaller both in length and width than the wild type secondary wing on the left. The mosaic 2c, developed at 20° C, shows a more extreme reduction

in size for the *honey notched wings*. The expression of the *notched wings* phenotype at the different rearing temperatures has not been influenced by the genetic constitution of the rest of the body. There appears to be complete autonomy for the temperature sensitive mutant *notched wings*.

Mosaic wings which have a mixture of both the wild type and the *honey, notched wings* phenotype are shown in Figures 3, 4, 5. It is clear from these drawings that there is a longitudinal striping pattern (see, for example, 3a, 3f, 4c, 4i). For some mosaics, the stripes ran the entire length of the wing (4a, 4c, 5a); for other mosaics, the stripe ran from the middle to the distal edge (3b, 3d, 4i, 5b). A longitudinal striping pattern has been reported for wings of *Drosophila* (Bryant, 1970).

For some mosaics, two distinct phenotypic areas were observed (3b, 3c); for others, three areas were observed. In 3a, 3e, and 3f, for example, there are two

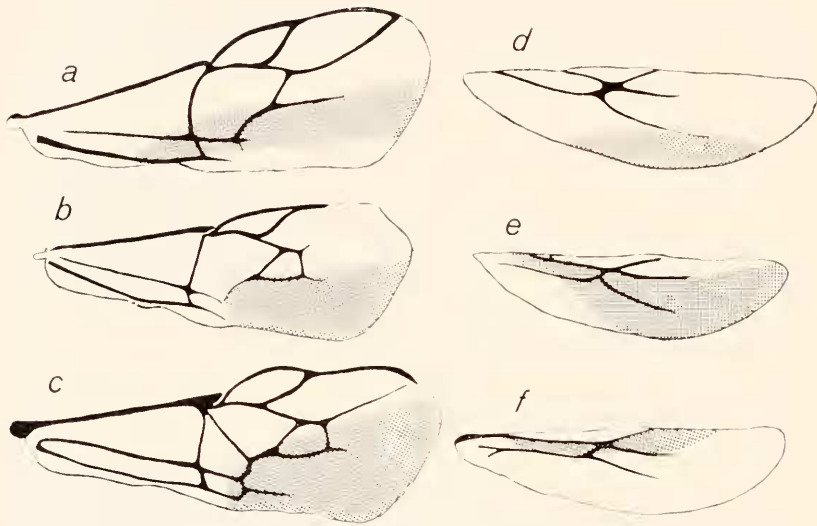


FIGURE 3. Wings mosaic for wild type (crosshatched) and *notched wings, honey*, reared at 30° C.

separate *honey, notched wings* areas separated by a wild type area; in 4b the wild type anterior and posterior edges are separated by a *honey, notched wings* region. In terms of clonal analysis, it would seem that these separate regions of the same phenotype arose from different progenitor cells in the developing wing disc.

Different proportions of the two phenotypes may be present in each mosaic wing. In 4a, about $\frac{1}{2}$ of the mosaic wing is wild type; in 3b about $\frac{1}{4}$; and, in 3f about $\frac{1}{4}$. In *Drosophila melanogaster* the relative sizes of mosaic patches have been used to establish the number of cells that initially were set aside for the adult wing during the first few hours of embryonic development. Perhaps in *Habrobracon* the relative sizes of the two phenotypes give an indication of the relative proportions of the two different cell genotypes that go to make up the presumptive wing disc.

Mosaic wings of different shapes and sizes were obtained from wasps reared at 20° and 25° C. These shapes and sizes were determined by the amount and posi-

tion of the *notched wings* genotype in the otherwise wild type wing (Figs. 4, 5). It is clear that where the *honey*, *notched wings* area appears, that area of the wing is smaller at the lower temperatures; and where the wild type area appears, the wing is larger. This is shown for the mosaic primary wings 5a, 5b, 5c, 5g. The mosaic wings are good examples of regional autonomy in response to temperature.

Ebony × *small wings*, *honey*

The four wings from a bilateral mosaic (Fig. 6a) show two left wings that are wild type and two right wings that are *small wings*, *honey*. The *small wings* are

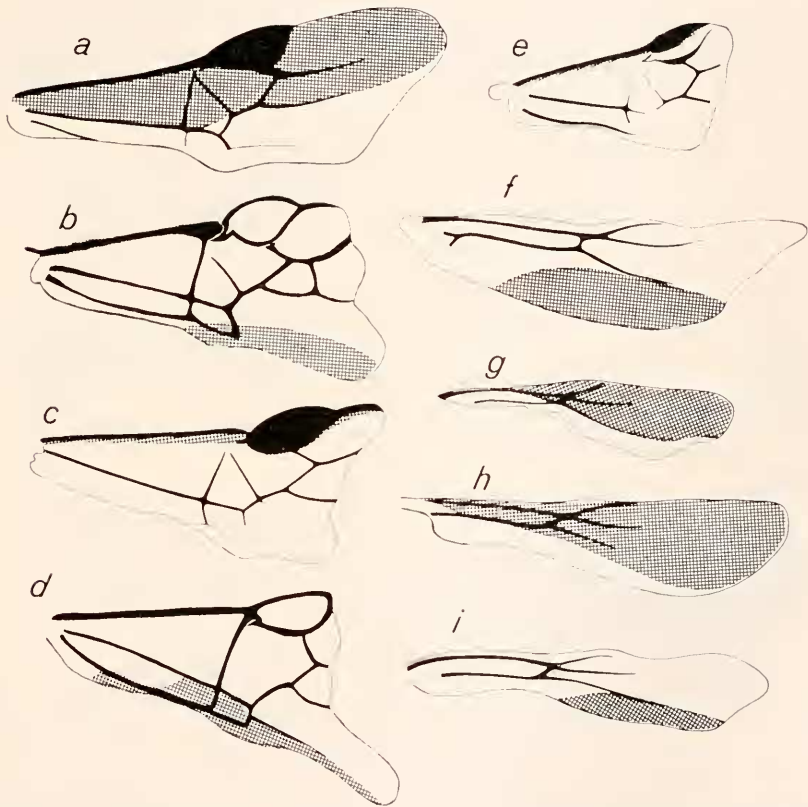


FIGURE 4. Mosaic wings for wild type and *notched wings honey*, reared at 25° C.

similar in shape, but smaller in length and width than the comparable wild type wings. For wings that are mosaic, differential growth of the wild type and mutant areas occurs. This is manifested by distortion of wing shape, wrinkles and curling of the wing. In the primary wing (Fig. 6b) where the anterior part is wild type and the posterior part is mutant, there is a downward bending of the wing. For the wings shown in Figs. 6c, 6e, 6f where the posterior part is wild type and the anterior part is mutant, there is a bending of the wing upward. The wrinkles that

appeared in mosaic wings were usually in a transverse direction and occurred in the wild type region of the wing (Figs. 6c, 6d, 6g). These wrinkles are more readily seen in the secondary wings (Figs. 6d, 6g). For secondary wings, there frequently occurred a curling so that the wing had a convex and a concave surface. Attempts to flatten such wings resulted in tears at the edges or in a folding-over of part of the wing.

The wild type and *small wings*, *honey* areas of the mosaic wing are clearly delimited. One can recognize the longitudinal striping pattern. The *small wings*, *honey* region shows the shorter microchaetae, more closely spaced, lying next to the wild type region with its longer microchaetae, more sparsely spaced.

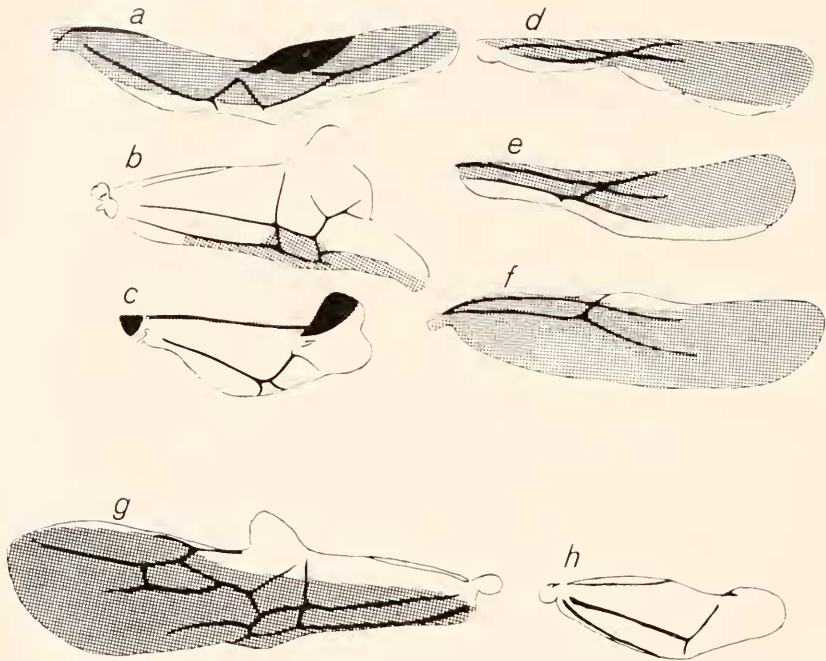


FIGURE 5. Mosaic wings for wild type and *notched wings honey*, reared at 20° C.

DISCUSSION

The wing mosaics obtained in *Drosophila* by Bryant (1970) arose as a result of X-ray-induced somatic crossing over in single cells. The size of the patches produced was related to the stage of development at which irradiation took place. Thus, in *Drosophila*, mosaicism was induced in an already formed disc or in one of a clump of cells destined to become a wing disc. Under these conditions a change in the genetic constitution of a single cell, which then proliferated, would give rise to a marked clone.

In mosaics of *Habrobracon*, nuclei of two different genotypes are already present at the start of cleavage and therefore the wing disc would already be a genetic mosaic at the time of its formation. Where the mosaic wing consisted of $\frac{1}{2}$ wild

type and $\frac{1}{2}$ honey, notched wings, the number of cells of each genetic type that went to make up the original wing disc is presumed to be equal. When $\frac{1}{2}$ of the wing area consisted of wild type tissue, then $\frac{1}{2}$ of the cells that made up the wing disc were wild type. Thus, one can estimate the proportion of cells, but not the number of cells, of each genotype that made up the original wing disc in *Habrobracon*.

In *Habrobracon*, some of the wing mosaics showed two longitudinal areas of the same phenotype separated by an area of different phenotype (Figs. 3a, 3e, 3f, 4b).

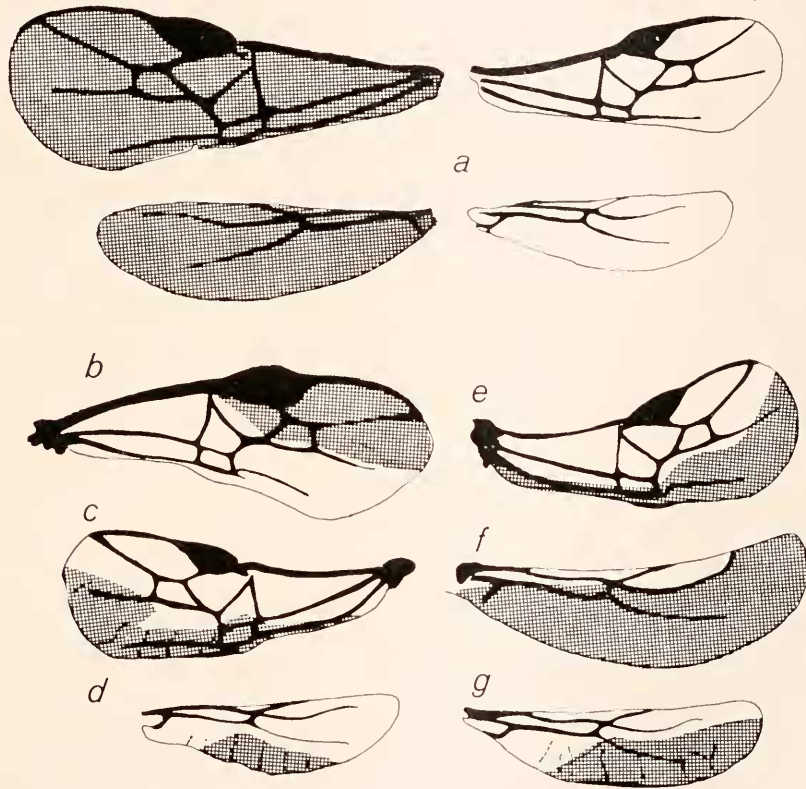


FIGURE 6. Wing mosaicism for wild type and *small wings honey*, reared at 30° C.

The explanation for this appears to be that the progenitor cells responsible for these separated regions were not contiguous in the imaginal disc. Thus, in these *Habrobracon* mosaics one might expect to get such multiple striping patterns while in *Drosophila* somatic crossover mosaics one would not expect to get them.

The mosaic wings found in *Habrobracon* showed a longitudinal striping pattern. This type of pattern has been found for *Drosophila* wings and indicates that oriented cell divisions are important in wing morphogenesis (Bryant, 1970). This interpretation for *Drosophila* can also be used to explain the patterns of wing mosaicism in *Habrobracon*.

Under 20° and 25° C rearing conditions the proportion of wild type and *notched wings*, *honey* tissue in the mosaic wings of adults is probably different from that of the wing disc. The *notched wings* mutant in *Habrobracon* appears to resemble the *vestigial* mutant in *Drosophila* in that it is temperature sensitive, showing loss of portions of wings. In *vestigial* it has been shown that this loss is due to cellular degeneration as the wing disc grows and differentiate (Fristrom, 1968, 1969).

Mosaic wings of different shapes and sizes were determined by the amount and position of the *notched wings* phenotype. There appears to be regional autonomy not only because the *notched wings* region develops independently of wild type but also because the growth of one part of the wing is not interfered with by the other part of the wing.

For the wild type—*small wings* mosaics, changes in wing shape also were found and depended upon the relative amounts and positions of the two phenotypes. Here, however, there appears to be an influence of the growth of each part of the wing on the other. The bends, wrinkles, and curls in the mosaic wings indicate that the *small wings* and the wild type parts are growing at the same time but at different rates. The bending of the wing downward is what one might expect when the wild type part is on the anterior surface, and upward when the wild type part is on the posterior surface. The wrinkles found primarily in a transverse direction and in the wild type part of the mosaic wing are indication also that wing growth from the imaginal disc occurs at different rates in *small wings* and wild type and also that this growth occurs in a longitudinal direction.

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SUMMARY

Habrobracon mosaics involving either wild type and *notched wings* or wild type and *small wings* were used to study mosaic patterns in wings. A longitudinal striping pattern was found for the wings of *Habrobracon*. This pattern is like that described for wings and other structures derived from imaginal discs in *Drosophila*. It indicates that cell divisions are preferentially oriented in certain directions in the developing disc (Bryant, 1970). Wings that were mosaic for *notched wings* showed regional autonomy with respect to temperature sensitivity. The different shapes and sizes of the mosaic wings were determined by the amounts and positions of the *notched wings* or *small wings* phenotype in them. Distortions in wing shape for wild type-*small wings* mosaics were related to concurrent growth and differential growth rates between the wild type and the *small wings* portions.

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